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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	04/29/1996	Final report: Sept1,95 - Feb 28,96

4. TITLE AND SUBTITLE
BIOEMULSIFIERS AND BIODEGRADATION OF ENVIRONMENTAL POLLUTANTS IN SOIL

5. FUNDING NUMBERS
C N68171-95-C-9106
PR WK2Q6C-7739-ENQ1

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
USARDSK-UK
EDISON HOUSE
223 OLD MARYLEBORNE ROAD
LONDON NW1 5th, UK

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT

DISTRIBUTION STATEMENT A
Approved for public release
Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

The effect of enhancement of biodegradation has been shown by application of a number of biosurfactants and bioemulsifiers *in vitro*. Among lipopeptides the highest activity has been found in experiments with 'open' form of lichenysin A. Lichenysin A itself and surfactin did not increase the biodestructive activity of microorganisms and moreover in most cases inhibited the PCB degradation. A new glucose lipid surfactant MM1 showed in several tests a very high bioavaliability enhancement. In a number of experiments the alkane-induced proteins from Gram-negative strains and polysaccharides from bacilli strains appeared to be efficient PCB emulsifiers improving the biodegradation capabilities of several microbial strains. It should be noted that generally bioemulsifiers and biosurfactants act very specifically, i.e. dependingly on strain and amount of these compounds used in experiment. Experiments set up with soil samples did not get a clear answer whether bioemulsifiers could critically enhance the rates of biodegradation processes *in situ*. Only with glucose lipid MM1 the more-less detectable positive effect, i.e. approx. 12% increase of PCB degradation comparing to control has been found.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Bioemulsifiers, biosurfactants, PCB biodegradation	23
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

19960520 101

DTIC QUALITY INSPECTED 1

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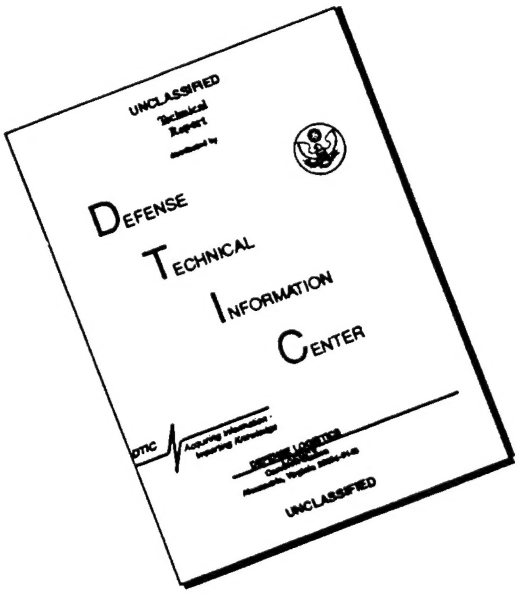

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BIOEMULSIFIERS AND BIODEGRADATION OF ENVIRONMENTAL POLLUTANTS IN SOIL

Final report

September 1, 1995 - February 28, 1996

by

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Contract #: N6868171-95-C-9106

The Research reported in this document has been made possible through its European Research Office of the U.S. Army. ~~This report is intended only for the internal management use of the Contractor and U.S. Government~~

Contents:

Summary

The effect of enhancement of biodegradation has been shown by application of a number of biosurfactants and bioemulsifiers *in vitro*. Among lipopeptides the highest activity has been found in experiments with 'open' form of lichenysin A. Lichenysin A itself and surfactin did not increase the biodestructive activity of microorganisms and moreover in most cases inhibited the PCB degradation. A new glucose lipid surfactant MM1 showed in several tests a very high bioavaliability enhancement. In a number of experiments the alkane-induced proteins from Gram-negative strains and polysaccharides from bacilli strains appeared to be efficient PCB emulsifiers improving the biodegradation capabilities of several microbial strains. It should be noted that generally bioemulsifiers and biosurfactants act very specifically, i.e. strongly dependingly on strain and amount of these compounds used in experiment. Experiments set up with soil samples did not get a clear answer whether bioemulsifiers could critically enhance the rates of biodegradation processes *in situ*. Only in one case, namely with glucose lipid MM1 the more-less detectable positive effect, i.e. approx. 12% increase of PCB degradation comparing to control has been found.

Keywords

Bioemulsifiers, biosurfactants, biphenyl, PCB, degradation

Introduction

Polychlorinated biphenyls (PCBs) represent a widely reported class of environmental pollutants. These compounds persist in the environment due in part to their low water solubility and high level of reclcitrance to bacterial and fungal degradation. Numerous reports have been published on the aerobic degradation of chlorinated biphenyls containing one to three chlorine atoms, with some reports on the development of mixed microbial enrichments and pure bacterial strains capable of aerobically degrading the highly chlorinated PCBs with four to six chlorine atoms. The natural recalcitrance of the highly chlorinated PCBs is based on steric hindrance of 2,3-dioxygenation due to chlorine moieties at either of these positions. Only several microorganisms- PCB degraders can overcome such steric incompatibility.

Another major factor limiting mineralization of PCBs, in addition to their natural recalcitrance, is the diffusion-limited availability of parent compounds and some of their metabolites in experimental systems. Functioning as emulsifiers or solubilizers, surface active agents produced by microorganisms can serve to increase surface areas and aqueous concentrations of poorly soluble compounds and thus potentially improve their accessibility to microorganisms by solubilization of substrates and/or promoting their transport across cell membranes.The exact mechanisms by which bioemulsifiers facilitate cellular uptake of hydrophobic compounds are not clearly understood and seem to be very special for different environmental systems.

The current study evaluates the effect of microbial bioemulsifiers: native and opened forms of lichenysin A, surfactin, glucose lipid MM1, protein like activator and extracellular protein extract from LB400, at concentrations below and above their critical micelle concentrations (CMC), on the enhancement of biological degradation of PCB congeners in flask and soil microcosm studies. Arochlor 1248 was uused in this study as model compound.

Results

1. Lichenysin A

We have been investigating the properties and production of surface active lipopeptide produced by subsurface isolated *Bacillus licheniformis* BAS50. This organism has been shown to produce a very

effective biosurfactant under both aerobic and anaerobic conditions. We have shown that this surfactant, lichenysin A, is slightly different to other known lipopeptide surfactants produced by *Bacillus* and is a mixture of structually close lipopeptides having 14 different straight and branched ß-hydroxy fatty acids as lipophilic moieties. The peptide moiety is composed of glutamic acid as the N-terminal amino acid, isoleucine as the C-terminal amino acid and asparagine, valine, leucine. Its structure has been determined:

C_{12}-C_{17} ß-hydroxy acid - Glu - Leu - Leu - Val - Asn - Leu - Ile

Purified lichenysin A achieves the critical micelle concentration (c.m.c.) at as low as 12 mg/l, characterizing the product as a powerful surface-active agent comparing favourably to other surfactants. Although nothing is known about the genes involved in the lichenysin production, proceeding from structural similarity to surfactin it is possible to postulate the same mechanism of its synthesis. Recently it was shown that the biosynthesis of surfactin is catalysed by a multienzyme system consisting of at least 4 enzyme components termed E_{1A}, E_{1B}, E_2 (subunits of surfactin synthetase), which catalyse the incorporation of seven amino acids in peptide chain, and E_3 (acyl transferase), which catalyses binding and transfer of the ß-hydroxy fatty acid substrate. The gene coding for E_3 enzyme has not yet been identified.

The application of both chemical and biological surface active agents for biodegradation can either affect negatively on microbes due to possible alteration of cell membrane functions.Taking this fact into consideration we determined the effect of surface active lipopeptides addition on bacterial growth. The antimicrobial activities of surfactin and lichenysin A were compared. A survey of the results is given in Table 1.The lipopeptide lichenysin A inhibited the growth of most of the bacteria tested on nutrient agar plates, but this inhibition was clearly less than that observed with surfactin.No growth inhibition by lichenysin A was detected for *Bacillus licheniformis*, *Bacillus subtilis* and *Rhodococcus globerulus*. To clarify the role of the polar groups on the antimicrobial activity, lichenysin A was treated with alkali to open the lactone linkage:

C_{12}-C_{17} ß-hydroxy acid - Glu - Leu - Leu - Val - Asn - Leu - Ile

↓NaOH

C_{12}-C_{17} ß-hydroxy acid - Glu - Leu - Leu - Val - Asn - Leu - Ile-COOH

OH

The open form of lichenysin A has distinctly decrease of microbial toxicity.

Table 1. Antimicrobial activity of surfactin and lichenysin A (15 µg) in agar diffusion tests.

Microorganism	Surfactin, halo diameter	Native lichenysinA halo diameter	Opened lichenysin A halo diameter
Acinetobacter calcoaceticus	+++	++	+
Alcaliges eutrophus	+++	++	+
Bacillus cereus	+++	-	-
Bacillus licheniformis	++	+	-
Bacillus subtilis	++	++	+
Escherichia coli	+++	++	++
Enterobacter	++	+	+
Pseudomonas fluorescens	++++	++	+
Pseudomonas proteofaciens	++	+	+
Rhodococcus globerulus	++++	-	-
Staphylococcus aiureus	++++	+	-

Maximum diameter of halos: -, <5mm; +, 6 to 7 mm; ++, 8 to 9mm; +++, 10 to 11 mm; ++++,>11 mm

The less polar lichenysin A peptide moiety comparing to that of surfactin and the opening of lacton ring structure appear to have an important influence on the decrease of the antimicrobial activities without sufficient inactivation of surface activity of lipopeptides.

The ability of both lichenysin A and 'open' lichenysin A to improve the biodegradation has been tested in microcosm *in vitro* studies vs different PCB degrading strains, namely ENV391, ENV 360, *Burkholderia cepacia* LB400, *Rhodococcus capsulatus* P6. Some of these strains have also been used for microcosm *in situ* biodegradation tests.

The studies on the growth of strain LB400 in minimal medium M9 contained biphenyl as sole carbon/energy sourcein the presence of 'open' lichenysin A has shown a significant increase of

biomass production comparing to control (**Fig.1**). The growth curve looks practically linear what

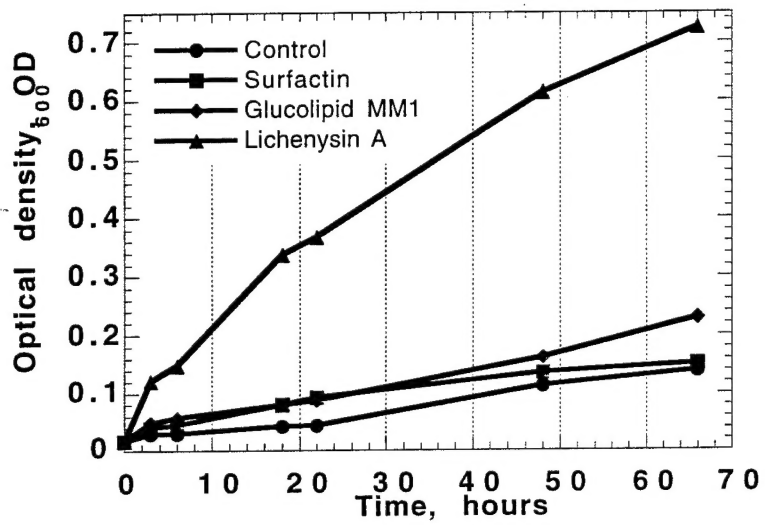
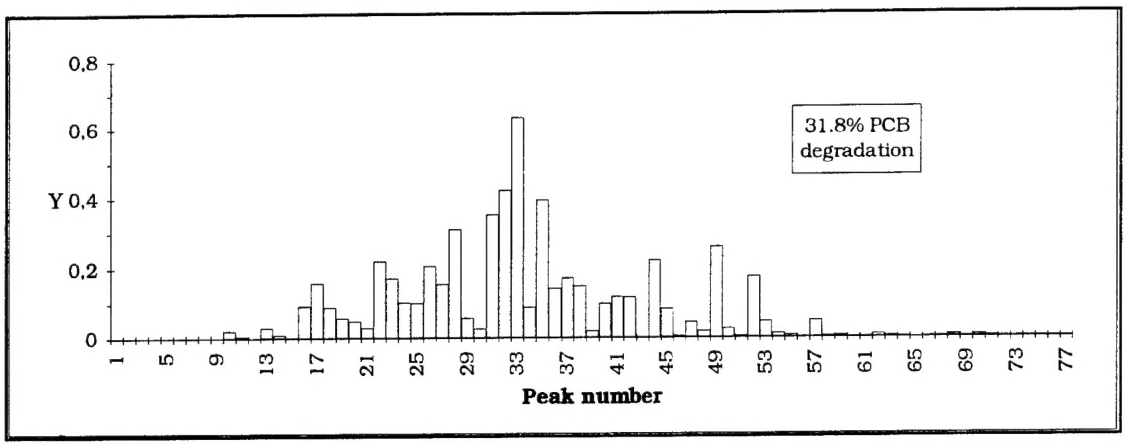


Figure 6. Enhancement of growth of LB400 in batch culture by addition of several bioemulsifiers. The cultures were grown on minimal M9 media supplemented with biphenyl as a sole carbon source. The concentration of added bioemulsifiers were of 120 mg/l.

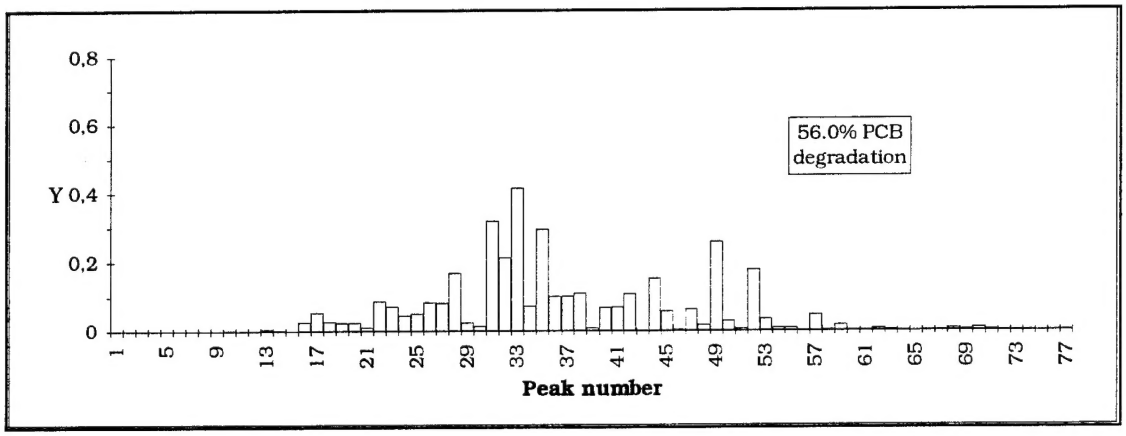
usually takes place in batch cultures with constant substrate flow like in so-called 'dialysis cultures' and in systems where the substrat-uptake rates are much higher comparing to solubility rates of substrate e.g. in solid-state fermentation processes. The slope of the curve in such culture types corresponds basically the substrate(s) bioavaliabiliy degree. Thus, the sufficient increase of biomass production in the presence of 'open' lichenysin A might be clearly explained by biphenyl solubility/bioavailability enhancement caused by addition of this emulsifier.

In microcosm studies 'open' lichenysin A added in concentration of 0.1% (ca. 70 CMC) enhanced the biodegradation capacity of strain LB400 from 31.8% to 56.0% in 24 hour assay and from 56.0% to 63.3% in 48 hour assay (Fig.2&3), ENV391 from 33.4% to 65.6% (Fig.4), ENV 360 from 38.3%

A) LB400 (24 hour assay)



B) LB400 (48 hour assay)



C) Killed Control - Arochlor 1248

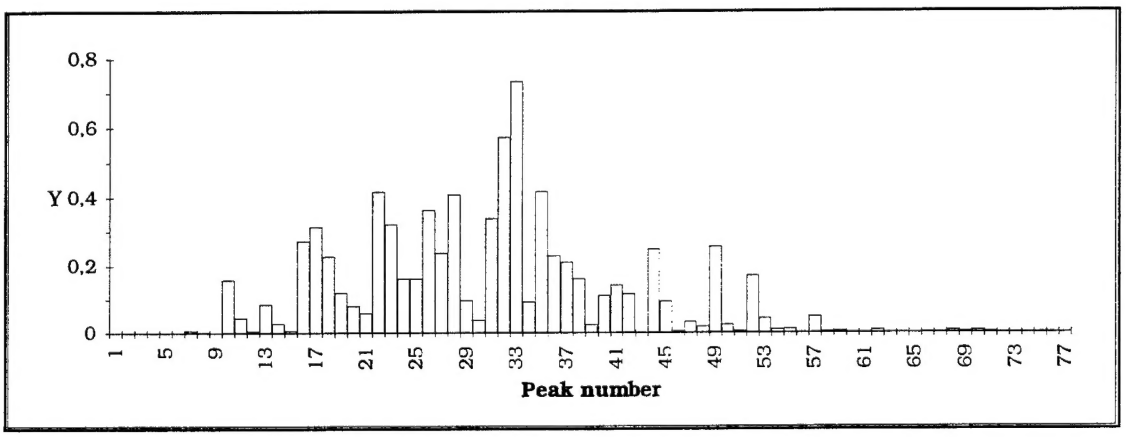
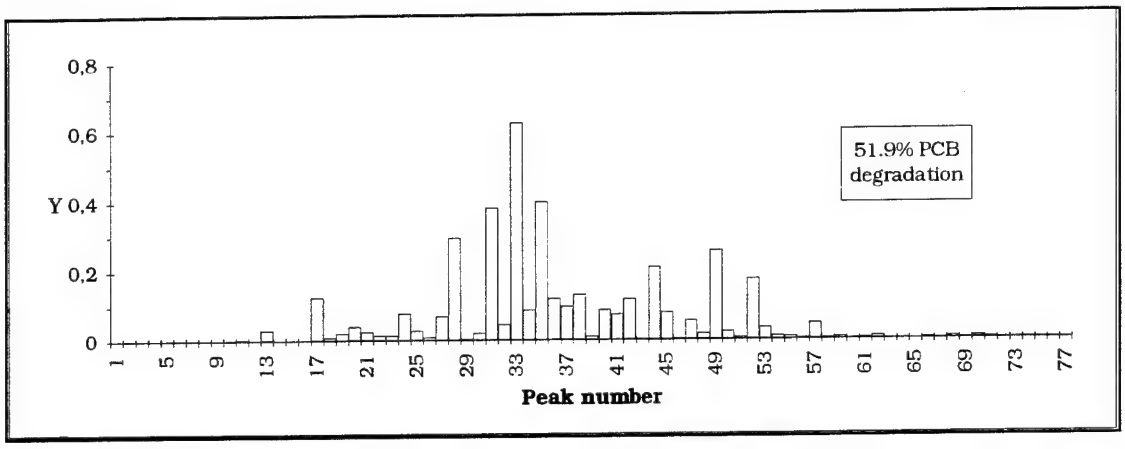
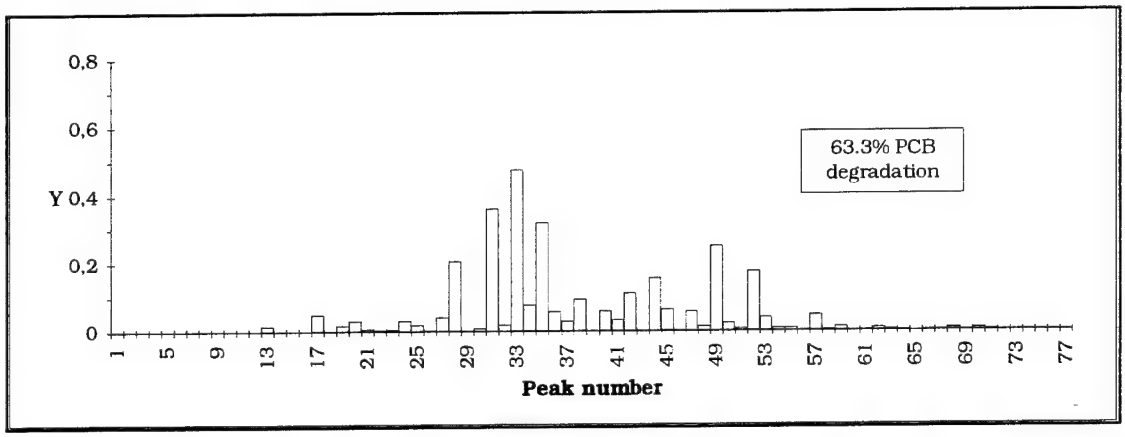


Fig.2 Arochlor 1248 degradation patterns of the strain LB400

A) LB400 + 0.1% 'open' Lichenysin A (24 hour assay)



B) LB400 + 0.1% 'open' Lichenysin A (48 hour assay)



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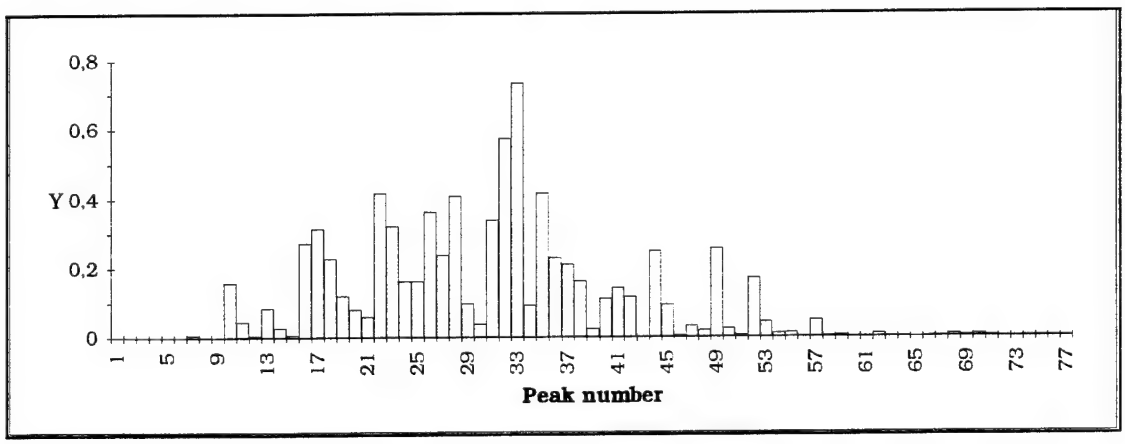
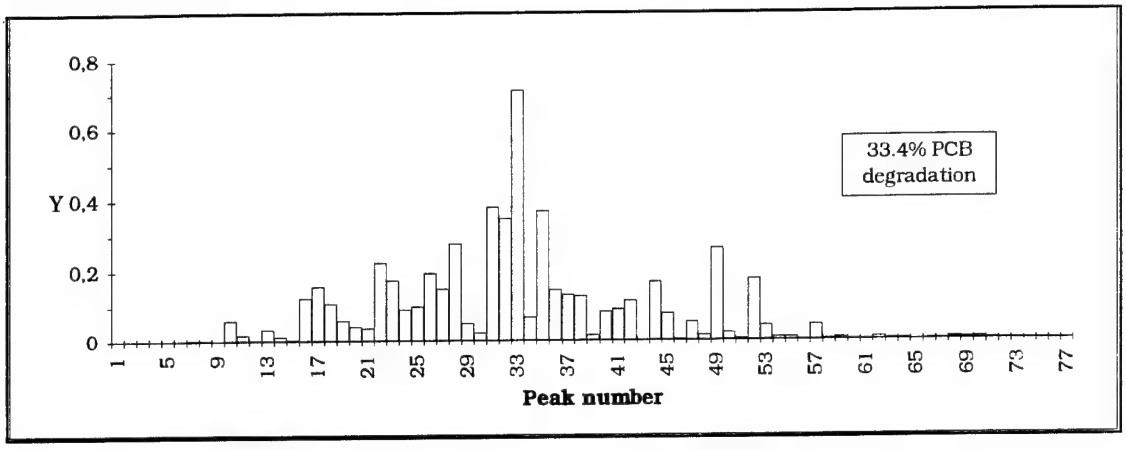
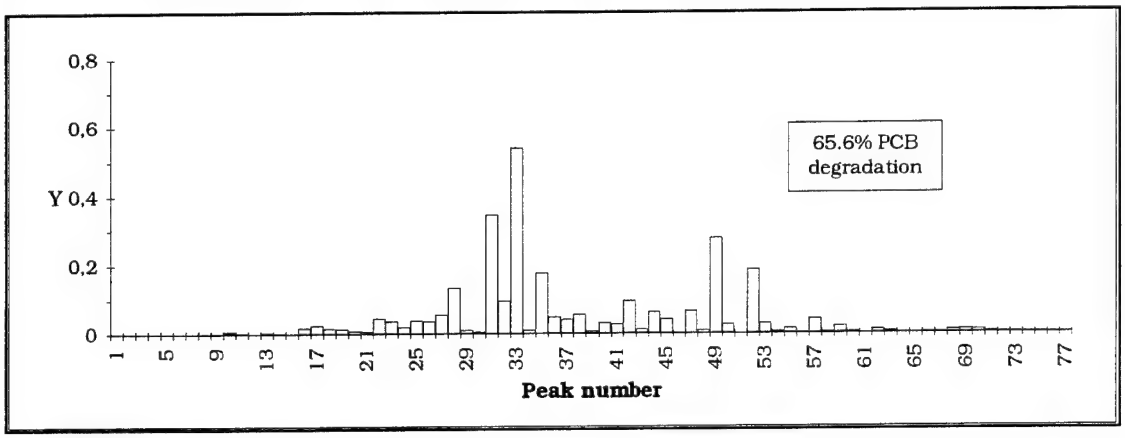


Fig.3 Arochlor 1248 degradation patterns of the strain LB400 in the presence of 'open' lichenysin A

A) ENV391 (24 hour assay)



B) ENV391 + 0.1% 'open' Lichenysin A (24 hour assay)



C) Killed Control - Arochlor 1248

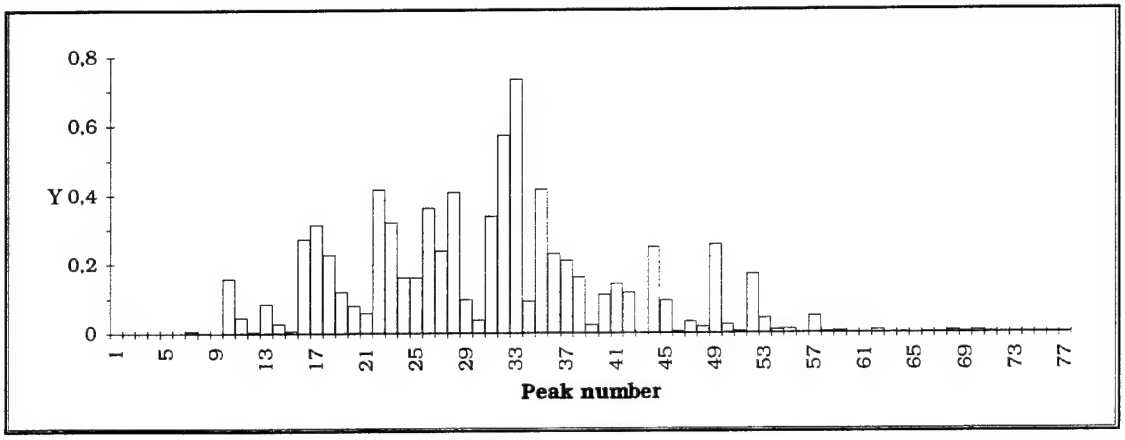


Fig.4 Arochlor 1248 degradation patterns of the strain ENV391 with and without addition of 'open' lichenysin A

to 57.4% (Fig. 5) and P6 from 30.8% to 41.5% (Fig. 6). In a test where a combination of strains ENV360 and ENV391 has been used the increase of PCB dedradation from 39.6% to only 45.1% (Fig.7) has been observed. It should be noted that in the latter case the concentration of biosurfactant was two times less than in previous experiment, i.e. 0.05% (ca. 35 CMC) and probably this amount of lichenysin A has been uptaken by membrane compartments of bacterial cells presented in that microcosm study in a duplicated population density.

For the 'opened' lichenysin A the inhibition was observed with only strain ENV360 at concentration of 0.1% (ca. 70 CMC). In that case degradation dropped from 38% in control to 17.7% (Table 2). In all other experiments with 'open' lichenysin A the percent of PCB degradation was remaining at the control level or higher.

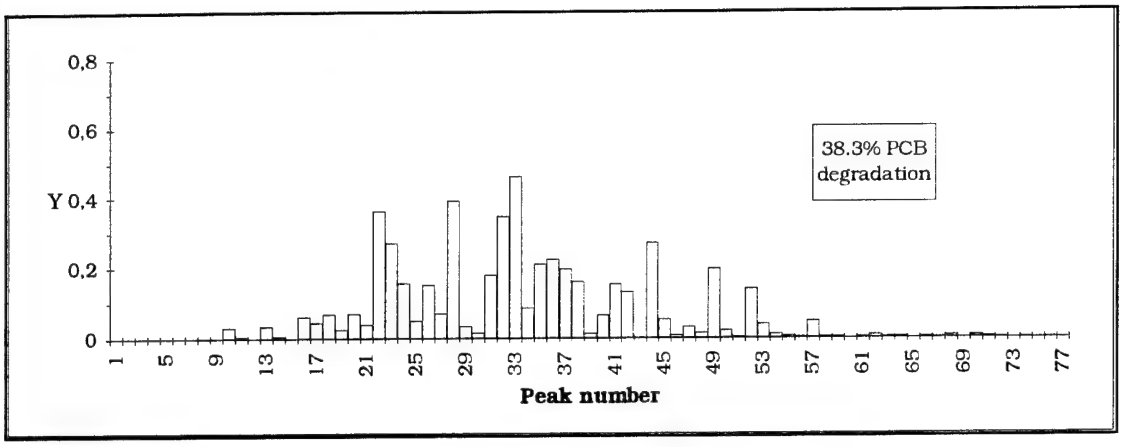
As shown in Table 2, the native lichenysin A did not seem to enhance the degradation of any tested microorganisms and almost caused an inhibitory effect on PCB-degraders, especially in higer concentrations (0.1%) e.g. degradation percent dropped from 28.7% (control) to 5.8%, from 30.8% to 0%, and from 38.3% to 23.3% in tests with strains LB400, P6, and ENV360 respectively. The addition of surfactin, an antimicrobial agent stronger than lichenysin A, caused the drastic inhibition of PCB degradation already at concentrations below CMC (0.001%) and completely stopped the PCB degradation for all tested strains at concentrations excessing 5 CMC (0.01%).

Degradation of PCB in the soil showed slight increase of PCB mineralization rates followed by the addition of 'open' lichenysin A in *in situ* microcosms. The rates of PCB degradation for ENV391+ENV360 were 57% and 60% with and without addition of 'open' lichenysin A, respectively (Fig.8, Table 3).

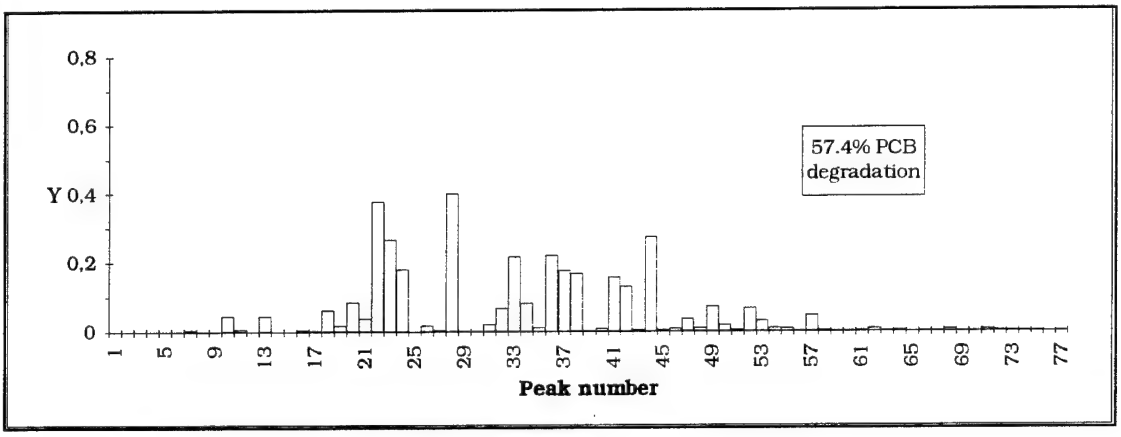
2. Glucose lipid MM1.

During screening for bioemulsifiers among marine, *n*-alkane-utilizing bacteria, low- and high-molecular surface-active substances were detected. The marine strain MM1, previously identified as *Alcaligenes* sp., was found to synthesize a novel glucose lipid that has not so far been cited in the

A) ENV360 (24 hours assay)



B) ENV360 + 0.1% 'open' Lichenysin A (24 hour assay)



C) Killed Control - Arochlor 1248

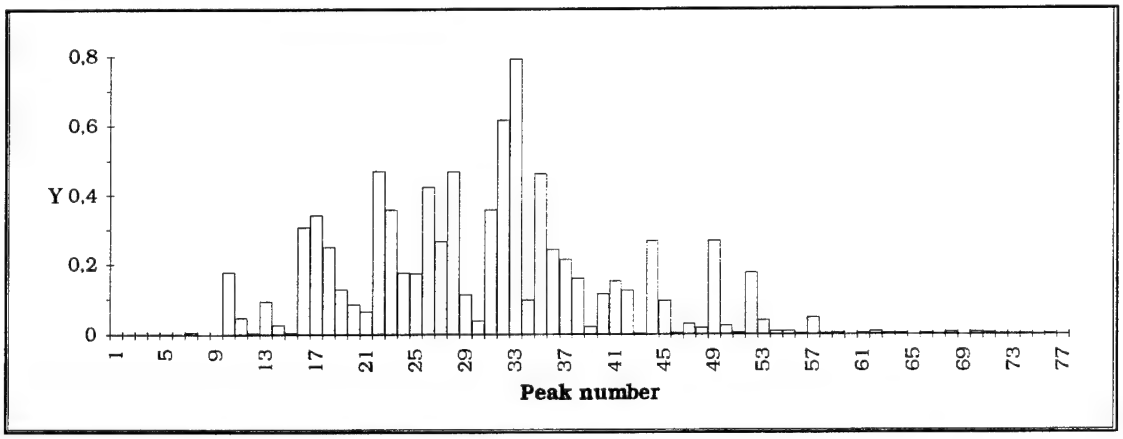
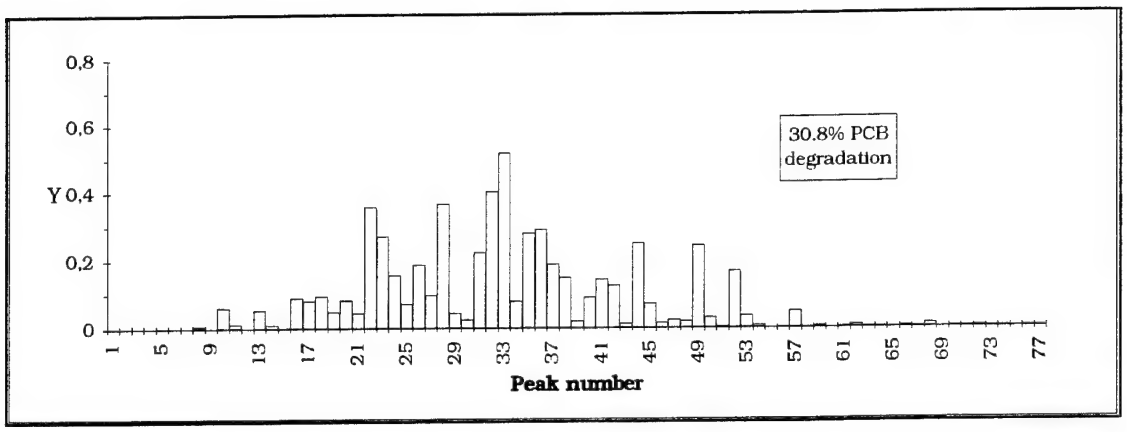
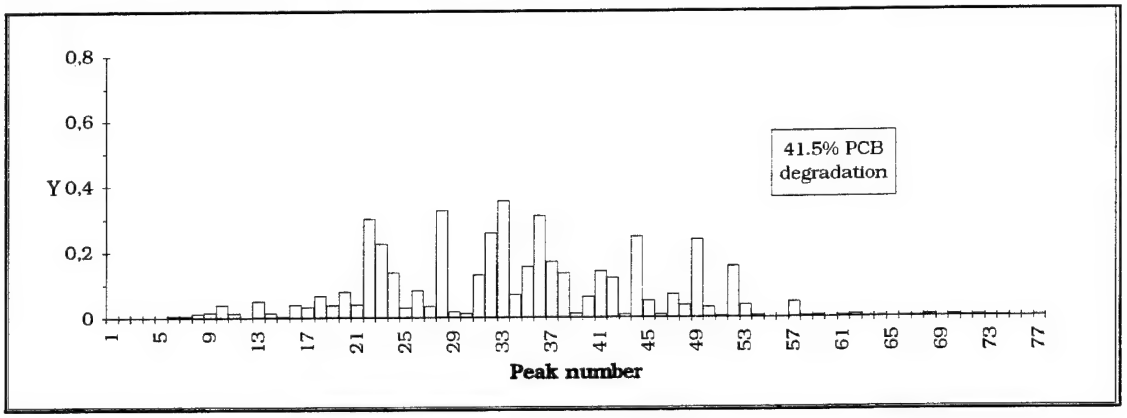


Fig.5 Arochlor 1248 degradation patterns of the strain EN360 with and without addition of 'open' lichenysin A

A) P6 (24 hour assay)



B) P6 + 0.01% lichenysin A ,'open' form (24 hour assay)



C) Killed Control - Arochlor 1248

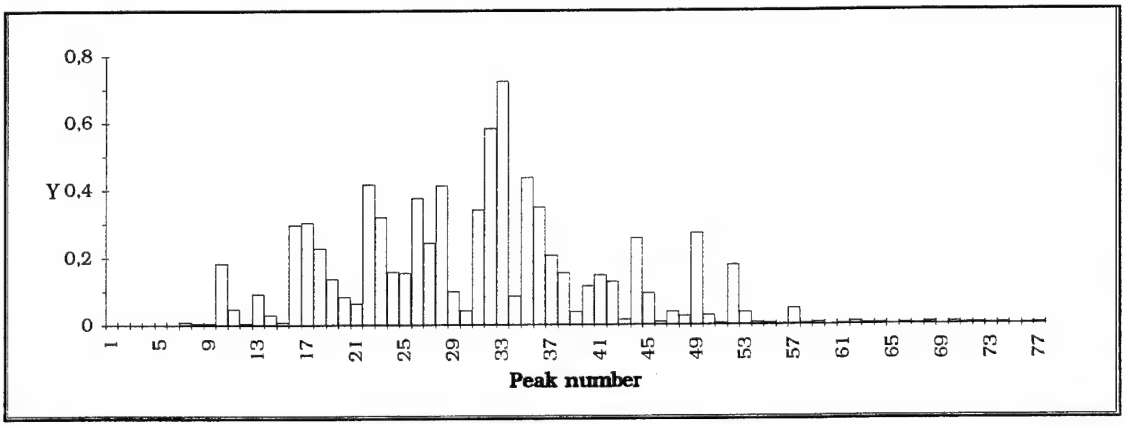
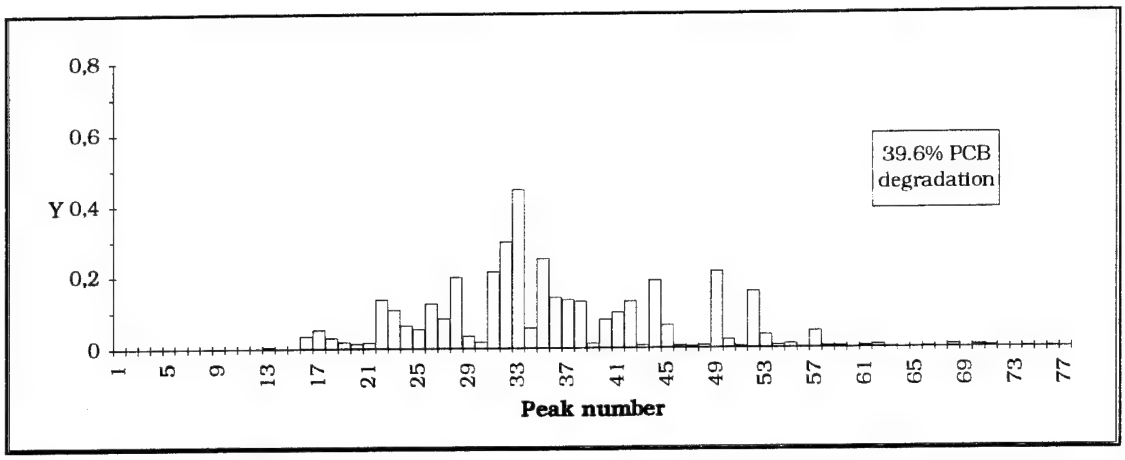
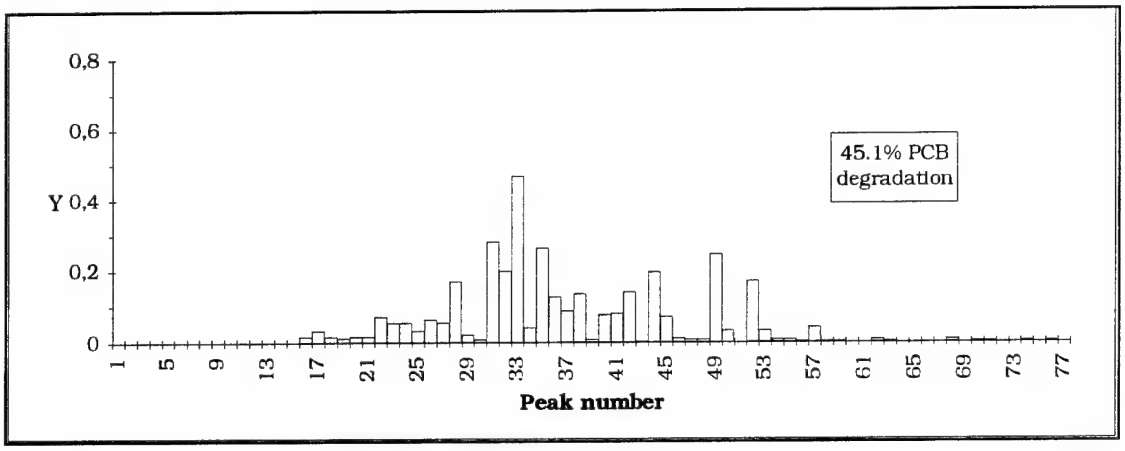


Fig.6 Arochlor 1248 degradation patterns of the strain P6 with and without addition of 'open' lichenysin A

A) ENV360 + ENV391 (24 hour assay)



B) ENV360 + ENV391 + 0.05% Lichenysin A ('opened' form)



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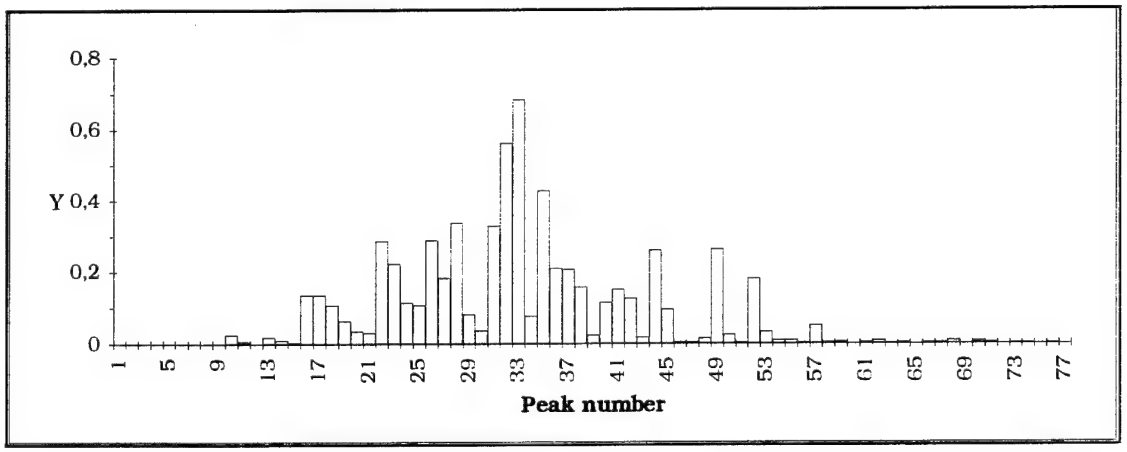


Fig.7 Arochlor 1248 degradation patterns of strains ENV360 and ENV391 with and without addition of 'open' lichenysin A

Table 2 Percent PCB Degradation Observed in 20 ppm Aroclor 1248 Assays with Various Strains

Surfactant	Concentration (%)	Assay Time	Percent PCB Degradation								
			LB400			ENV 391		P6		ENV 360	
			Exp 1*	Exp 2	Exp 3	Exp 1	Exp 2	Exp 1*	Exp 2	Exp 1	Exp 2
-	0%	24 hr	28,7	31,8	46,1	33,4	32,2	30,8	20,4	38,3	41,3
-	0%	48 hr	-	56,0	-	-	-	-	-	-	-
Glucolipid MM1	0,001%	24 hr	35,1	-	-	-	-	34,9	-	49,0	-
	0,01%	24 hr	20,6	-	-	-	-	13,0	-	54,0	-
	0,1%	24 hr	0	-	-	-	-	32,8	-	57,4	-
PA fraction	0,001%	24 hr	28,3	-	-	-	-	22,3	-	50,9	-
	0,01%	24 hr	13,0	-	-	-	-	32,9	-	44,3	-
	0,1%	24 hr	17,5	-	-	-	-	21,8	-	51,6	-
LB400 protein fraction	0,001%	24 hr	25,7	-	-	-	-	36,4	-	48,1	-
	0,01%	24 hr	41,6	-	-	-	-	30,4	-	47,5	-
	0,1%	24 hr	50,2	-	-	-	-	29,3	-	43,4	-
Lichenysin A "open" form	0,001%	24 hr	19,2	-	-	-	-	5,0	-	28,6	-
	0,01%	24 hr	22,2	-	-	-	-	41,5	-	50,5	-
	0,05%	24 hr	-	52,6	-	-	-	-	-	-	-
	0,1%	24 hr	44,4	51,9	-	65,6	-	27,7	-	17,7	-
	0,1%	48 hr	-	63,3	-	-	-	-	-		-
Lichenysin A	0,001%	24 hr	20,6	-	-	-	-	12,5	-	29,9	-
	0,01%	24 hr	7,4	-	-	-	-	2,0	-	36,6	-
	0,1%	24 hr	5,8	-	-	-	-	0	-	23,3	-
Surfactin	0,001%	24 hr	21,3	-	-	-	-	6,6		36,9	-
	0,01%	24 hr	0	-	-	-	-	0		8,7	-
	0,1%	24 hr	0	-	-	-	-	0	-	3,9	-
Polysaccharide 20	0,001%	24 hr	-	-	51,2	-	37,0	-	43,0	-	24,4
	0,01%	24 hr	-	-	56,5	-	35,5	-	32,6	-	32,5
	0,1%	24 hr	-	-	37,6	-	27,5	-	39,4	-	37,8
Polysaccharide 29	0,001%	24 hr	-	-	51,7	-	37,1	-	40,8	-	36,9
	0,01%	24 hr	-	-	51,8	-	23,1	-	30,4	-	43,4
	0,1%	24 hr	-	-	55,4	-	48,0	-	34,0	-	35,0

A (-) indicates that these samples were not done

*Degradation results for most of the samples in these columns are an average of two assays.

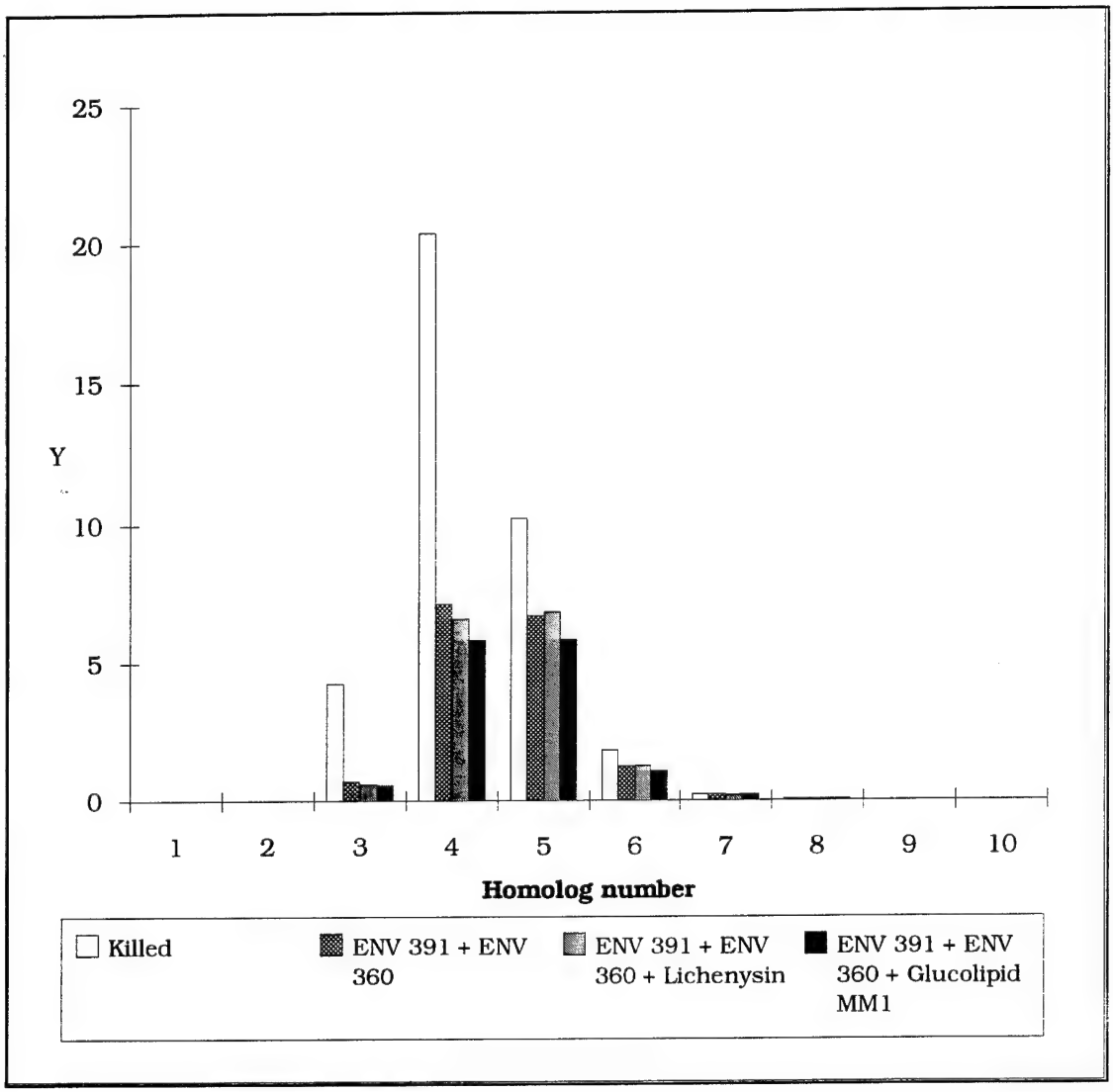


Fig. 8 PCB homolog degradation patterns ENV360 and ENV391 with and without addition of glucose lipid MM1 and lichenysin A

Table 3

Summary of Results from Vial Study

Vial Number	Soil Source (from *in situ* soil microcosms - 6 week time point)	Amount of Soil (g-dry)	Bacterial dosing/ Biosurfactant Amendment[1]	PCB Concentration (mg/kg)	Total Percent Degradation
1	Killed Microcosm	2.15	None, acid to pH 3 / None	36.9	-
2	Intrinsic Control	1.95	None / None	21.6	42
3	Bioaugmented Microcosm (ENV 391 + ENV 360)	1.89	ENV 391 + ENV 360 / None	15.8	57
4	Bioaugmented Microcosm (ENV 391 + ENV 360)	1.88	ENV 391 + ENV 360 / "new" open Lichenysin (40% NaCl), 0.02% w/v	15.3	58
5	Bioaugmented Microcosm (ENV 391 + ENV 360)	1.82	ENV 391 + ENV 360 / open Lichenysin used in Microcosm study; 0.02% w/v	15.8	58
6	Bioaugmented Microcosm (ENV 391 + ENV 360)	1.95	ENV 391 + ENV 360 / Glucolipid MM1, 0.01% w/v	13.3	64
7	Glucolipid MM1 (0.01% w/w, Week 1 and 4) / Bioaugmented Microcosm (ENV 391 + ENV 360)	2.04	ENV 391 + ENV 360 / None	14.7	60
8	Glucolipid MM1 (0.01% w/w, Week 1 and 4) / Bioaugmented Microcosm (ENV 391 + ENV 360)	2.00	ENV 391 + ENV 360 / Glucolipid MM1, 0.01% w/v	13.4	64
9	Lychenysin (0.02% w/w, Week 1 and 4) / Bioaugmented Microcosm (ENV 391 + ENV 360)	1.90	ENV 391 + ENV 360 / None	19.1	48
10	Lychenysin (0.02% w/w, Week 1 and 4) / Bioaugmented Microcosm (ENV 391 + ENV 360)	1.98	ENV 391 + ENV 360 / open Lichenysin used in Microcosm study, 0.02% w/v	17.7	52
11	Lychenysin (0.1% w/w, Week 1 and 4) / Bioaugmented Microcosm (ENV 391 + ENV 360)	1.97	ENV 391 + ENV 360 / None	17.6	52
12	Lychenysin (01% w/w, Week 1 and 4) / Bioaugmented Microcosm (ENV 391 + ENV 360)	1.84	ENV 391 + ENV 360 / open Lichenysin used in Microcosm study, 0.1% w/v	18.7	49

[1]ENV 391 was added to a final concentration of 1.5×10^8 cells /ml and ENV 360 was added to 3.4×10^7 cells /ml.

Note: Biphenyl was added at 0.05% at time 0 and day 7, vials were flushed with oxygen at time 0 and day 7. Vials were shaken for a total of 14 days.

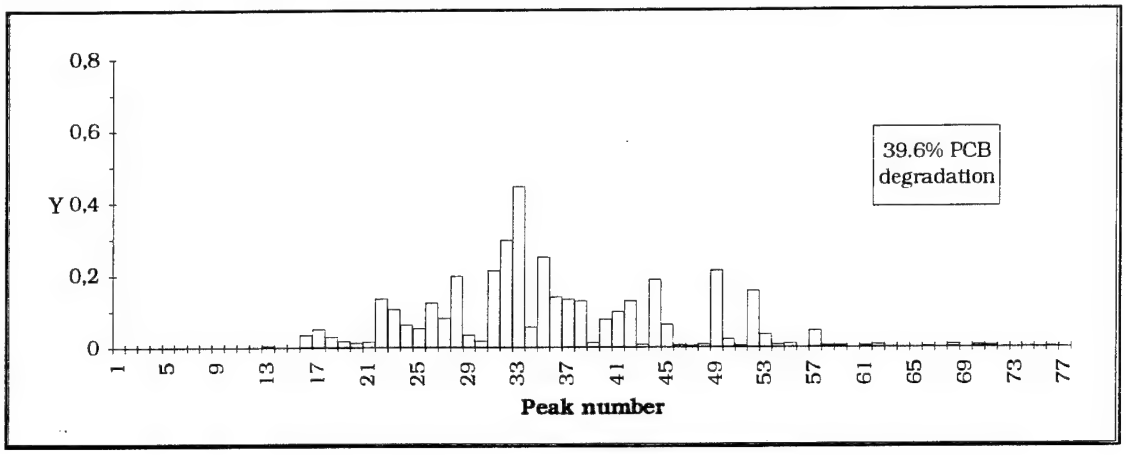
literature. Nuclear magnetic resonance spectroscopic and mass spectrometer studies led to the identification of a glucose lipid consisting of four residues of ß-hydroxy acids varying in lengh sizes between C_8 and $C_{10,}$ which are linked together by ester bonds. The lipophilic moiety is coupled glycosidically with C-1 of glucose: **Glucose--C_{10}--$C_{8\text{-}10}$--$C_{8\text{-}10}$--C_9COOH**. The glucolipid reduced the surface tension from 72 mN/m to 30 mN/m while the minimum interfacial tension towards *n*-hexadecane was lowered to values smaller than 5 mN/m, characterizing the product as a powerful surface active agent. Further identification by using 16S rRNA gene sequence analysis showed that the strain MM1 possessed a 16s rRNA gene sequence most similar (approximately 89-92%) to 16S rRNA gene sequences of microorganisms of the genera *Halomonas* and *Deleya* (family *Halomonadaceae*).

Application of glucose lipid MM1 revealed a strong positive effect on PCBs degradation.First tests with glucolipid MM1 showed a stimulation of LB400 growth in batch culture with biphenyl. After 68 hours of batch experiment the amount of LB400 biomass was ca. two times more comparing to control (**Fig. 1**).

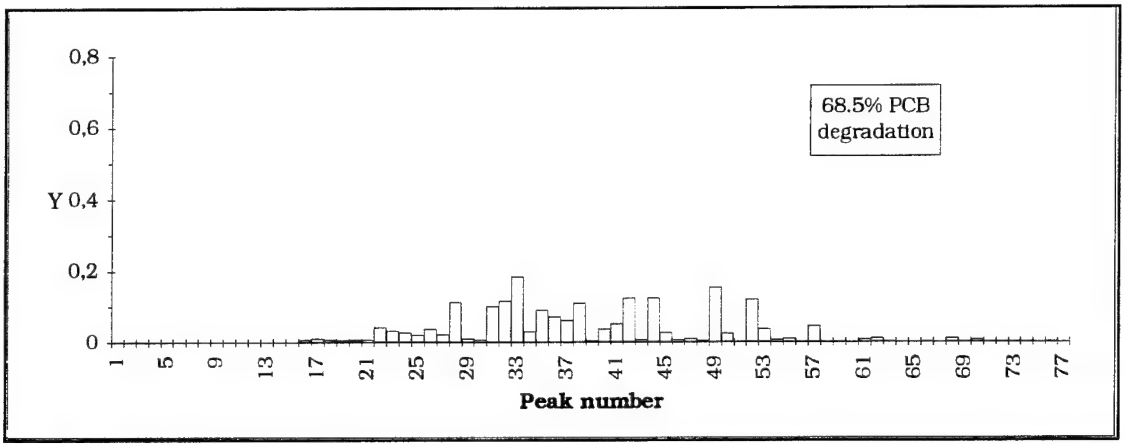
Glucolipid isolated from marine strain MM1 caused the most detectable improving effect on degradation of Arochlor 1248 by strain ENV360 (Table 2). In this case the dedradation increased from 38.3% to 57.4%. The positive effect has not been detected in the tests with the strains P6 and LB400. However in an experiment where the combination of ENV360 and ENV391 was used glucolipid MM1 stimulated the biodegradation capacity sufficiently, from 39.6% to 68.5% within 24 hours of incubation (Fig. 9).

Experiments set up to elucidate whether stimulating effect takes place *in situ* , showed the glucolipid MM1 is only the bioemulsifier which application caused the increase of biodegradation in the soil (Table 3). The low increase of PCB degradation (ca. 12%) could be explained by high degradation activity of indigenous microorganisms and definitively at this high level the increment of biodegradation due to bioemulsifier effect is not as detectable as in vial studies. At the other hand the soil is very heterogenic system and soil particles, different minerals and organic matters may attach emulsifiers critically dropping their activity.

A) ENV360 + ENV391 (24 hour assay)



B) ENV360 + ENV391 + 0.01% Glucolipid MM1



C) Killed Control - Arochlor 1248

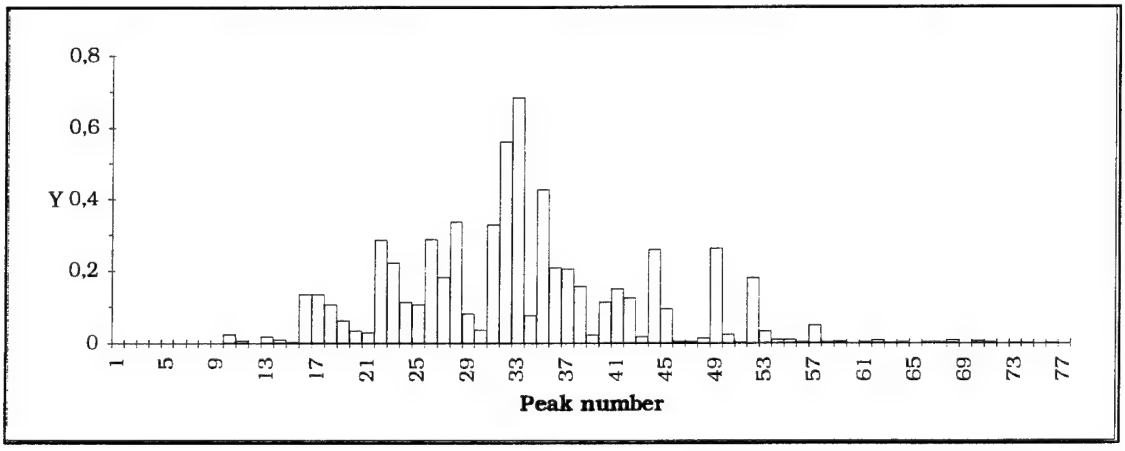


Fig.9 Arochlor 1248 degradation patterns of strains ENV360 and ENV391 with and without addition of glucose lipid MM1

3. Alkane-induced protein-like emulsifiers

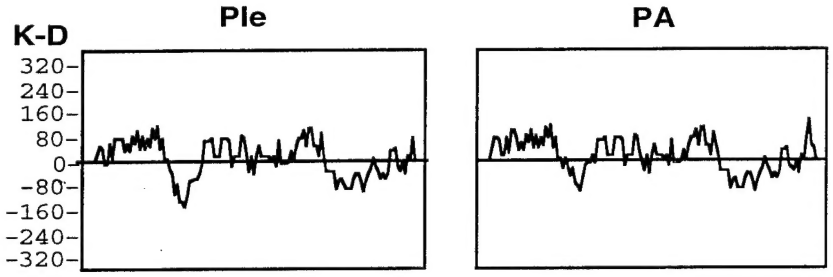
The *P. aeruginosa* PG201 and *Burkholderia cepacia* LB400 strains produced a number of ammonium sulfate-precipitable extracellular proteins when cultivated in the media containing *n*-hexadecane or biphenyl (in case with LB400) as a carbon source. PA proteins with a molecular mass of roughly 16 kDa represented up to 90% of the precipitated on StrataClean resin extracellular protein fraction of the PG201, as judged from SDS-PAGE analysis (Fig. 2, 2nd interim report). Interestingly, that the production of PA protein was greatly increased by cultivation of PG201 in an excess of nitrogen. In case of LB400 we found that proteins with molecular masses between 25 and 71 kDa were represented as an abundant fraction of extracellular proteins. However, two proteins with molecular masses of 16.5 and 18 kDa correspond to putative PA protein analogs were also detected.

To confirm a hypothesis that LB400 produces the protein-like activator(s) we checked out the presence in the chromosomal DNA of this strain of sequences similar to PA-encoding gene (*pra*) from *P. aeruginosa* using DNA-amplification with specific oligonucleotide primers. The 520 bp PCR-generated product was obtained and this product exactly corresponds to *pra* gene size. From the analysis of the DNA sequence with the GeneWork program (release 2.45) we found only one open reading frame (ORF), termed *ple* (protein-like emulgator), and deduced amino acid sequence of the Ple revealed a very high homology to PA protein:

```
Ple   MKSIKSLPSF GGLALCLSVS SMARALTITP VNSGFTAPGT ISVSSPASLN   50
PA    MKSIKSLPSF AALALCLSVS SMASAAATITP VNSAFTAPGT ISVSSPASLN   50

Ple   LPVTCNITFK GKTAADGSYA SIDSVTVSGS NTLCSVPQMT GLPWKLTVSS  100
PA    LPVTCNITFK GKTAADGSYA SIDSVTVSGS NTLCSVPQMT GLPWKLTVSS  100

Ple   TTAGKVDGVG FKILSSTCGP STVNGSWSNA TNTLSASNQS LAGNCKINSL  150
PA    TTAGKVDGVG FKILSSTCGP STVNGSWSNA TNTLSASNQS LAGNCKINSL  150

Ple   SVKPTPAFVV NP                                          162
PA    SVKPTPAFVV NP                                          162
```

Analysis of the hydrophobicity profiles suggests the presence in both PA and Ple proteins two

slightly hydrophobic helices separated by two stretches of hydrophilic sequences, showing that these proteins are amphophilic and could have emulsifying activities. The plots were made by the program Gene Work (release 2.45) and represent the averages of a residue-specific hydrophobicity.



Application of PA protein isolated from P.aeruginosa PG201 for in vitro biodegradation experiments showed an enhancement of biodegradation activity in only one case, i.e. vs strain ENV360, from 38.3% to 51.5%. This positive influence of PA-protein has been shown even for pretty low concentrations (0.001% or 2μM) of this emulsifier.For other tested strains neither enhancing nor inhibiting actions of PA-potein have been detected (Table 2).

Alkane-induced extracellular proteins from LB400 appeared to be stimulating for biodegraders ENV360 and LB400. The percent of degradation was increased from 30.8 to 48.1% and from 28.7 to 50.2% respectively.

These data are very promising from the point of view of further work in direction to establishment of new microbial strains able to produce bioemulsifiers and degrade pollutants. Protein biosynthesis processes comparing to biosynthesis of complex substances such as lipopeptides or glucose lipids are more amenable for genetic manipulations and might be expressed easily by strains-biodegraders rather than more complex genetic loci determining biosynthesis of non-protein-like compounds.

4. Polysaccharides from *Bacillus licheniformis* strains BNP20 and BNP29

Several high molecular compounds of microbial origin such as polysaccharides and lypopolysaccharides were characterized as emulsifiers. To investigate the positive effect of polysaccharide addition on PCB degradation we used two polymers isolated from the culture of two

different strains *Bacillus licheniformis*. As was shown by infrared spectroscopy, both products contain glucose and glucuronic acid residues and have molecular weight about 50-70 KDa. Polysaccharide from *B.licheniformis* BMP29 slightly increased the biodegradation capacity of strain LB400 and ENV391, from 46,1% to 55.4% and from 32.2% to 48.0% respectively. No stimulation was detected in experiments with strain ENV391, but in case with P6 the activity of latter increased twice, from 20.4% to 40.8% (Table 2) even at low polysaccharides concentration value (0.001%). Polysaccharides from *B.licheniformis* BMP20 had a dramatic effect on the same strain, namely P6, and very low enhancement influence on LB400. The degradation values increased from 20.4 to 43.0% and from 46.1 to 56.5%, respectively.

Discussion

The toxicity characteristics of some surfactants seem predictable due to their tendency to disrupt cell membranes and therefore to cause the lytic effects. Ionic surfactants have generally been associated with irreversibly altering cell membranes.Direct contact of surfactants with or incorporation into cell membranes are significant factors when considering the disruptive effects of this agents. The different responses observed for different microorganisms might be due to differing compatibility requirements between the surfactants and the different cell wall compositions of Gram-negative and Gram-positive microorganisms. Our data concerning antimicrobial activity of surfactin and lichenysin A showed that Gram-positive bacteria are more susceptible to these lipopeptides than Gram-negative organisms. The same effect was observed in PCB degradation experiments. The'open' lichenysin A which is less toxic for microbial cells revealed the highest values of stimulation of PCB degradation in spite of its lower surface activity than native lichenysin A. Thus, the ratio between surface activity and toxicity of tested bioemulsifiers should be an important consideration in the selection of suitable agents to enhance biodegradation activity.

The effect of enhancement of biodegradation has been shown by application of a number of biosurfactants and bioemulsifiers *in vitro*. Among lipopeptides the highest activity has been found in

experiments with 'open' form of lichenysin A. Lichenysin A itself and surfactin did not increase the biodestructive activity of microorganisms and moreover in most cases inhibited the PCB degradation. A new glucose lipid surfactant MM1 showed in several tests a very high bioavaliability enhancement. In a number of experiments the alkane-induced proteins from Gram-negative strains and polysaccharides from bacilli strains appeared to be efficient PCB emulsifiers improving the biodegradation capabilities of several microbial strains. It should be noted that generally bioemulsifiers and biosurfactants act very specifically, i.e. strongly dependingly on strain and amount of these compounds used in experiment. Experiments set up with soil samples did not get a clear answer whether bioemulsifiers could critically enhance the rates of biodegradation processes *in situ*. Only in one case, namely with glucose lipid MM1 the more-less detectable positive effect, i.e. approx. 12% increase of PCB degradation comparing to control has been found. The difficulties to provide the correct experiments with such complex system as soil are caused by (i) high initial level of degradation activity of indigenous microorganisms annealing the positive effect of surfactants and (ii) high absorbance activity of soil particles, presented, for instance, by clay minerals, different organic matters etc., that may attach emulsifiers and decrease their activity significantly.

For further researches concerning application of bioemulsifiers *in situ* it seems to be interestingly to combine the ability to degrade pollutants and produce bioemulsifiers simultaneously within one bacterial strain. In that case the cell may produce the bioemulsifier providing itself with higher concentration of substrates locally in closest environment in the soil.

Two publications based on the results obtained during the work at this project are in preparation. Selected data will be reported at International Biodedradation Symposium (June 29-July 2), Mallorca, Spain

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